
**Supplementary file S4**

**Figure S4: Hydroxychloroquine increases Cathepsin G and MMP12 activity.** (A) Cathepsin G (n=4, *P*=0.03; Cuzick’s test for trend) and (B) Macrophage elastase (MMP12) activity in whole cell lysates of HM605 infected J774A.1 murine macrophages (n=4, *P*=0.04 Cuzick’s; *P*<0.05 Dunnett’s test). (C) Cathepsin G (n=4, */#P*<0.05; ANOVA) and (D) MMP12 activity in response to *E. coli* infection (n=4, *P*=0.44, ANOVA).

Methods S4: Intra-macrophage protease activity was determined using enzyme-specific substrate degradation assays. Briefly, J774A.1 murine macrophages were seeded in triplicate to 24-well plates. Following 2h infection, cells were washed three times, incubated with media containing 20μg/mL gentamicin for 1h to remove extracellular bacteria, then treated for a further 3h with media containing HCQ (0-10μg/mL) or dimethylsulfoxide (DMSO) vehicle control. Cells (at 6h) were washed with sterile PBS and lysed with sterile 50mM HEPES, 50mM NaCl, 0.1% Triton-X100 for 30 min on ice. Aliquots of lysate (100μL) were transferred to white-walled, clear bottom 96-well plates (Corning, Tewksbury, MA, USA) and specific substrates for each enzyme added: N-succinyl-Ala-Ala-Pro-Phe p-nitroanilide for Cathepsin G (final concentration 1mM) and N-methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide (0.85mM) for Macrophage elastase (MMP12) as per[1-3]. Plates were incubated for 24h in the dark and OD_{410nm} quantified using a Sunrise™ microplate reader.

Enzyme activity was calculated using the Beer-Lambert equation [4]. A linear relationship between enzyme activity and optical densitometry was confirmed by co-incubating serial dilutions of purified Cathepsin G (0.5-20nM; Sigma, Poole, UK) and Macrophage elastase (0.5-2.5nM (Enzo Life Sciences, Exeter, UK)) with their relevant substrates for 24h in the dark then optical densitometry determined previously detailed.